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Application Note

Detection and Quantification of Leached Protein A from Eshmuno® A Resin

Using Cygnus F610 Protein A Mix-N-Go™ ELISA Kit

Introduction

Protein A affinity chromatography is the standard capture method for purification of monoclonal antibodies. As some of the protein A ligand co-elutes with the antibody, this impurity must be removed from the product during subsequent purification steps. Consequently, leached protein A is routinely quantified during downstream purification.

Project Definition

The Cygnus Protein A Mix-N-Go™ ELISA Kits are a new generation of protein A detection kits. They enable testing of high product concentration samples, have improved accuracy and sensitivity, shorter time to results and samples require no heating or centrifugation steps as compared to the predecessor Cygnus Protein A F400 ELISA kit.

This study was performed to confirm suitability of the Cygnus Protein A Mix-N-Go™ ELISA F610 kit (Cygnus F610 kit) for detection and quantification of the smaller, recombinant protein A ligand, similar to that of Eshmuno® A resin. The second part of the study confirmed the accuracy, precision and range of quantitation of this assay for measuring levels of leached Eshmuno® A protein A ligand in bioprocessing samples.

Feasibility Testing: Accuracy and Quantitation Limits

Phase I testing compared the theoretical concentration of an Eshmuno® protein A ligand dilution series with the measured concentration using the Cygnus F610 kit. The ligand was pre-diluted to 10 ng/mL in Cygnus I600 Mix-N-Go™ sample diluent and used to prepare a series of 2-fold dilutions that corresponded to the concentrations of the Cygnus F610 protein A standard. These diluted samples were tested in duplicate as recommended in the Cygnus F610 kit. The data were analyzed using a 4-parameter logistic fit for curve fitting. This test was performed on 2 different days to assess reproducibility.

Results

Figure 1 shows the curve of the mean measured concentration of Eshmuno® protein A ligand being close to congruent to the Cygnus F610 kit standard curve over its full range.

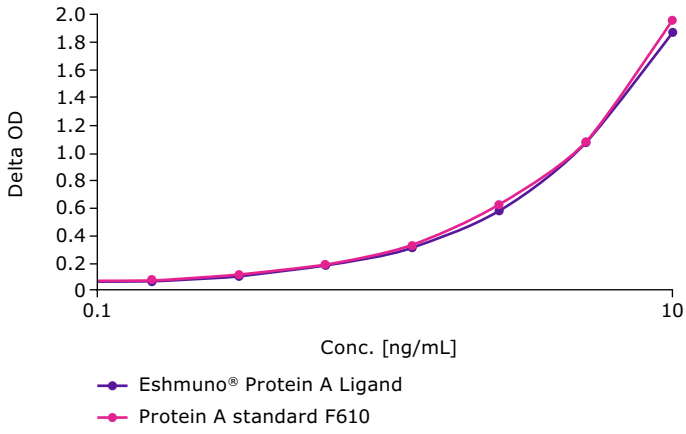

Figure 1.

Comparison of protein A ligand dilution series with Cygnus F610 kit standard curve.

The quantification data, Table 1, shows the lowest concentration of the Eshmuno® protein A ligand resulted in the highest variability in quantified values with coefficient of variation (CV%) exceeding the acceptance criteria of ±20% of the nominal concentration at the lower limit of quantitation (LLOQ) specified in the kit instructions on day 1. Repeatability exceeded 20% of the CV on day 2 and for the overall CV of both days. Excluding the lowest ligand concentration, the test had a 96.8% nominal recovery in mean using protein A standard from the kit.

Table 1.

Quantification of Eshmuno® A protein A ligand in the Cygnus F610 kit. Red values indicate quantified values with CV% exceeding the ±20% acceptance criteria.

	Day 1			Day 2					
Theoretical Conc [ng/mL]	Quant. result	Average result [ng/mL]	CV [%]	Quant. result	Average result [ng/mL]	CV [%]	Overall Average result [ng/mL]	Overall CV [%]	Overall nominal recovery [%]
10	9.542	9.444	1.5	9.087	9.488	6.0	9.466	3.6	94.7
	9.345			9.888					
5	5.141	4.957	5.2	5.076	4.985	2.6	4.971	3.4	99.4
	4.773			4.893					
2.5	2.462	2.497	2.0	2.237	2.390	9.1	2.443	5.8	97.7
	2.531			2.543					
1.25	1.245	1.252	0.7	1.185	1.150	4.3	1.201	5.4	96.1
	1.258			1.115					
0.625	0.631	0.624	1.6	0.591	0.626	7.9	0.625	4.7	100.0
	0.617			0.661					
0.313	0.313	0.302	5.2	0.263	0.280	8.3	0.291	7.1	92.9
	0.291			0.296					
0.156	0.090	0.098	10.9	0.135	0.161	22.8	0.129	33.1	82.9
	0.105			0.187					

Standard curves generated over multiple days of feasibility testing were compared (Figure 2).

Although the Cygnus F610 kit manual listed the LLOQ as 0.1 ng/mL, our results demonstrate the accuracy of the quantification may be found lower at the lowest standard concentration (0.156 ng/mL) with two of ten values (marked as red) exceeding the acceptance criteria of ±20% of the nominal concentration. This observation may vary depending on the performing lab and chosen regression model as several curve fitting routines are applicable for the kit.

These results confirmed that the Cygnus F610 kit accurately and reliably detects Eshmuno® A protein A ligand with a quantification range of 10–0.313 ng/mL.

Phase II of this study explored qualification of the Cygnus F610 kit assay for use with the samples that had been purified with Eshmuno® A resin.

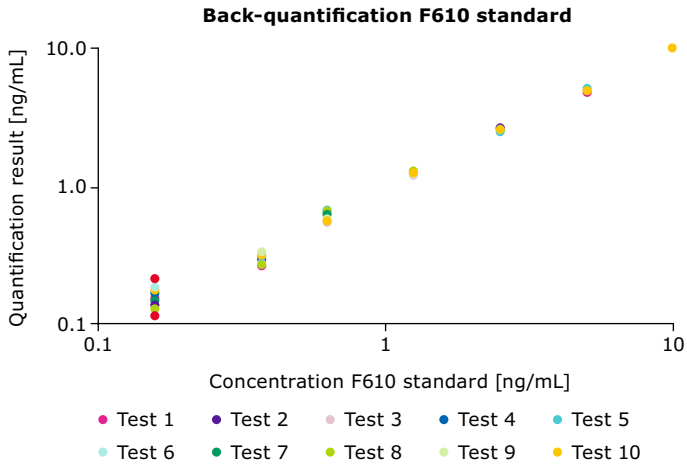

Figure 2.

Accuracy of quantification of the Cygnus F610 Protein A Mix-N-Go™ standard.

Qualification of the Cygnus F610 Protein A Mix-N-Go™ ELISA Kit for use with the Eshmuno® A resin

The Cygnus F610 kit was qualified for use with Eshmuno® A resin using samples collected from a mAb purification. For assay qualification, accuracy (spike recovery) and precision (repeatability) were assessed with 3 lab scale, in-process samples over 2 days; Table 2 summarizes the sample information. Successful qualification should deliver accuracy of +/-20% nominal and precision of CV <15% for each sample.

Table 2.

Sample information.

Sample	Buffer Conditions	Approximate pH	Approximate product concentration [mg/mL]
Eshmuno® A Elution	Elution Buffer (0.1 M acetic acid, pH 3) neutralized to pH 5 with 2 M Tris	5.0	26.4
Post-CEX	50 mM sodium acetate, pH 5 with gradient to 0.5 M NaCl, then neutralized to pH 7.5 with 2 M Tris	7.5	12.0
Post-AEX	25 mM Tris, pH 7.5	7.5	7.3

Qualification Test Procedure

The range of the dilutions to be tested for each sample were determined in a previous experiment over a larger range of dilutions (data not shown) which revealed very low protein A ligand concentrations for both Post-CEX and Post-AEX samples. The decision was made to include sample dilutions slightly above the recommended product concentration of 5 mg/mL product, if no inhibition is observed. The Post-CEX sample showed precipitation with the denaturation buffer and some inhibition when used undiluted; this was overcome by a 1:2 dilution in the sample diluent provided in the kit.

For each sample, 4 serial dilutions and spiked samples were tested. An aliquot of each initial sample dilution was spiked with the Eshmuno® protein A ligand for a spike concentration of 5 ng/mL.

Serial dilutions of unspiked and spiked samples were prepared, treated with denaturation buffer, and tested in duplicate according to the instructions of the Cygnus F610 kit.

Sample testing was repeated on a second day to assess reproducibility.

Qualification Results

Table 3 summarizes the accuracy and precision testing results; raw data is provided in the appendix (Table 4).

Table 3 shows that for the Eshmuno A Elution sample, measured concentration of protein A ligand was 151.5 and 172.2 ng/mL on days 1 and 2 respectively. Overall precision of all dilutions over both days resulted in CV of 9.7%. Protein A ligand levels in the Post-CEX and Post-AEX samples were quantified at the highest dilutions. However, while above the LOD, the ligand levels in these samples were below the LLOQ. Quantifiable dilutions of these samples showed a slight tendency to overestimate protein A levels, which may be due to the high concentration sample matrix. These effects can be overcome by sample dilution, however, due to the already low protein A levels in these samples no further dilution could be performed. The precision of the analysed Post-CEX sample dilutions from day one and day two had an overall CV of 4.6%. The precision of the analysed Post-AEX sample dilutions from day one and day two had an overall CV of 7.6%.

For the spike recovery, accuracy in the Eshmuno® A Elution sample was 87% on day 1 and 101% on day 2. For the Post-CEX sample spike recovery had an accuracy of 107% on day 1 and 119% on day 2 while the Post-AEX sample had an accuracy of 108% on day 1 and 118% on day 2.

Based on this testing, all three samples passed the acceptance criteria of +/-20% nominal for spike recovery on both days and a CV of less than 15% for day-to-day reproducibility.

Table 3.

Quantification and spiking results of all 3 samples from the different mAb purifications steps from 2 different days.

Sample	Day	Sample dilution	Quantification result dilution [ng/mL]	Quantification result sample [ng/mL]	Reported result sample [ng/mL]	Day-to-day-test-variation CV [%]	Quantification result spiked sample dilution [ng/mL]	Average spike recovery [%]	Overall spike recovery [%]
Eshmuno® A Elution	1	50	2.849	142.5	151.5	9.7	7.272	88	87
		100	1.495	149.5			3.972	99	
		200	0.742	148.4			1.807	85	
		400	0.414	165.6			0.878	74	
	2	50	3.235	161.8	172.2		8.784	111	101
		100	1.616	161.6			4.411	112	
		200	0.872	174.4			2.068	96	
		400	0.478	191.2			1.009	85	
Post-CEX	1	2	1.018	2.04	2.0	4.6	6.703	114	107
		4	0.505	2.02			3.026	101	
		8	<LOQ	N.A.			1.432	N.A.	
		16	<LOQ	N.A.			0.729	N.A.	
	2	2	1.108	2.22	2.2		7.796	134	119
		4	0.543	2.17			3.147	104	
		8	<LOQ	N.A.			1.598	N.A.	
		16	<LOQ	N.A.			0.710	N.A.	
Post-AEX	1	1	1.363	1.36	1.4	7.6	7.165	116	108
		2	0.665	1.33			3.474	112	
		4	0.353	1.41			1.560	97	
		8	<LOQ	N.A.			0.800	N.A.	
	2	1	1.635	1.64	1.5		8.542	138	118
		2	0.752	1.50			3.499	110	
		4	0.368	1.47			1.684	105	
		8	<LOQ	N.A.			0.798	N.A.	

Conclusion

This work has confirmed that the Cygnus F610 Protein A Mix-N-Go™ ELISA Kit is suitable for assessing levels of leached protein A from bioprocessing samples following purification on Eshmuno® A resin. Assay performance is summarized below:

- Feasibility testing demonstrated a range of 0.313–10 ng/mL for accurate detection of the Eshmuno® A protein A ligand using the Cygnus F610 kit.
- Accuracy and precision of the ELISA was confirmed using multiple samples from a 3-step antibody purification process using Eshmuno® A resin.

Before using this kit as part of a routine testing with other purification products or processes, it is highly recommended that assay performance is verified using representative samples.

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